

Please amend the application as follows:

In the specification:

Replace the paragraph on page 18, lines 21-24 with the new paragraph below.

The inhibiting effect of the compounds of the present invention was estimated using the assay described in: Dirk Hendriks, Simon Scharpé and Marc van Sande, Clinical Chemistry, 31, 1936-1939 (1985); and Wei Wang, Dirk F. Hendriks, Simon S. Scharpé, The Journal of Biological Chemistry, 269, 15937-15944 (1994). The essential aspects of the assay system described in these references are set forth below.

Add the following new paragraphs immediately following the paragraph on page 18, lines 21-24.

Principle

Carboxypeptidase U (CPU) acts on the substrate hippuryl-arginine. Arginine is cleaved off from the substrate, resulting in the formation of hippuric acid. Hippuric acid is then detected by means of HPLC.

Materials

Chemicals

ProCPU was purified from human plasma according to Clinica Chimica Acta, 292, 25-40, (2000). Hippuryl-arginine and thrombin were from Sigma, thrombomodulin was from American Diagnostica, 2-Methylhippuric acid was from Aldrich, PPACK*HCl was from Alexis, ethyl acetate was from Fisher Scientific Int Company and all other reagents and solvents were from Merck.

HPLC system

The system employed for measurement of generated hippuric acid comprised a high precision pump model 480, auto sampler Gina 50, auto sampler rack 5805.9210, UV-detector UVD 1706 and degasser GT-102 (Gynkotec, München, Germany) with Chromeleon software (version 6,00) and a Sperisorb C18, 5 ODS, (150 mm x 4.6mm) column using 85% KH_2PO_4 (10 mM, pH 3.5, adjust pH with 10% H_3PO_4) and 15% acetonitrile as the mobile phase.

Activation of proCPU

Thrombin

Thrombin (10 U) was dissolved in 5 mM CaCl_2 (1 mL). This solution was further diluted with dilution buffer to give a 12 nM thrombin solution.

Thrombomodulin

A 1 mL vial of 430 nM thrombomodulin (30 U thrombomodulin) was diluted with dilution buffer to give a 48 nM thrombomodulin solution.

PPACK

PPACK*HCl (5 mg) was dissolved in distilled H₂O (1 mL). This solution was further diluted with buffer to give a 20 μ M PPACK solution.

Dilution buffer

The dilution buffer consisted of Hepes 20 mM, CaCl₂ 5 mM, Tween 80 0.01%, pH7.4.

Inactivated plasma

Citrated human blood was centrifuged at 20000 x g for 20 minutes at 4°C. The plasma was then incubated for 12 hours at 56°C.

ProCPU

ProCPU was diluted in 50 mM Hepes buffer (ph 7.4) to give a CPU activity of about 0.5-1.5 mU per sample in the assay.

Activation of proCPU

A solution of thrombomodulin (48 nM, 100 μ L) and a solution of proCPU (100 μ L) were added to a solution of thrombin (12 nM, 100 μ L). The mixture was incubated for 10 min at room temperature.

A solution of PPACK (20 μ M, 100 μ L) was added and the mixture was incubated for 10 minutes at room temperature. Inactivated

plasma (100 μ L) was added and the resulting mixture was put on ice.

Assay for carboxypeptidase U activity
Substrate

Hippuryl-arginine (503 mg) was dissolved in 50 mM HEPES buffer (50 mL, pH 7.4) to give a 30 mM solution of hippuryl-arginine. The solution was sonicated prior to use.

Internal standard

2-Methylhippuric acid (291 mg) was dissolved in 99.5% EtOH (25 mL) and distilled H₂O was then added to give a total volume of 100 mL. The solution was diluted further with 4 volumes of 25% EtOH before addition to assay.

Carboxypeptidase U activity assay

Substrate (30 mM, 40 μ L) was added to each vial. A solution of a carboxypeptidase U inhibitor of different concentrations (5 μ L) or vehicle 5 μ L) was then added to the vials. The assay was started by adding proCPU (5 μ L) every 10 or 15 seconds to the vials, one at a time. The mixture was then incubated for 30 minutes at 37°C. The assay was stopped by adding HCl (1M, 50 μ L) to one vial at a time every 10 or 15 seconds. Internal standard (10 μ L) and ethyl acetate (300 μ L) were then added to each vial. The sample mixtures were mixed by turning the vials upside down 30 times. The vials were then centrifuged for 1

minute at 1000 x g. Two hundred (200) μ L of the upper layer (the ethyl acetate phase) was transferred to HPLC-vials and the solution was evaporated to dryness using a stream of nitrogen. The samples were redissolved in mobile phase (75 μ L) and analyzed in the HPLC system. For calculation of the effect of carboxypeptidase U inhibitor, the enzyme activity was determined in the presence of increasing concentrations of inhibitor.